Supplementary information:

Anti-biofilm activity of garlic extract loaded nanoparticles

Vallerinteavide Mavelli Girish, Hongying Liang, Jennifer T. Aguilan, Joshua D. Nosanchuk, Joel M. Friedman and Parimala Nacharaju

Methods:

MRSA strain:

Clinically characterized MRSA strain 6524 was obtained from Dr. Nosanchuk's lab and has been utilized extensively in prior studies. [1, 2] An overnight culture of this strain in tryptic soy broth (TSB; MP Biomedicals, Santa Ana, CA) was set up at 37 ˚C on a rotary shaker (Thermo Fisher Scientific, Waltham, MA) with agitation at 150 rpm. Growth was monitored by measuring the optical density at 600 nm using a microplate reader (BioTek, Winooski, VT).

Preparation of garlic extract:

The major initial sulfur compound present in the intact garlic bulbs is alliin (S-allylcysteine sulfoxide). Alliin is converted into allicin (diallyl thiosulfinate) by the enzyme allinase, within seconds of disruption of a garlic clove. [3, 4] Allicin is unstable and transforms into various disulfides, ajoenes and vinyldithiins with time. [4] These products also exhibit antimicrobial activity. [5] Isolation or synthesis of individual components is cumbersome. Therefore, we decided to use total garlic extract (GE) for our initial studies. The composition of the garlic components depends on the method of extraction. [6] We used the following method to prepare a GE containing allicin as the major product. Fresh garlic cloves obtained from a local market were ground to a fine paste in a food processor without adding any solvent. To 65 g of this paste 50 ml of 20% ethanol was added and stirred at room temperature for 2 h. This mixture was centrifuged at 1900 g for 20 min at 4 ˚C. The supernatant was separated and stored in aliquots at -80 ˚C.

Characterization of allicin in garlic extract:

The GE was fractionated on a Vydac reverse phase c18 column (4.6 x 250 mm) equilibrated with solvent A (0.1% trifluoroacetic acid [TFA]) using a Shimadzu HPLC system, LC-20AB (Columbia, MD). The bound analytes were eluted using a linear gradient of 0-50% solvent B (100% acetonitrile in 0.1% TFA) at 1 ml/min flow rate. The elution was monitored at 210 nm. The allicin peak was identified using an allicin standard (LKT Laboratories, Inc., St. Paul, MN) run under similar conditions. Fractions under this peak together with the standard were subjected to mass spectrometric analysis to confirm the mass and identity of allicin. The amount of allicin in the GE was calculated from area under the peak followed by comparison with the known amount of the standard.

Mass Spectrometry analysis of allicin from garlic extract and commercial standard:

HPLC fractions of GE containing allicin in 20% acetonitrile/0.1% TFA and 1.2 nmol/µl of allicin standard in 1:3 dilution with 50% acetonitrile/ 0.01% TFA were separately analyzed by Electrospray Ionization Mass Spectrometry **(**ESI-MS) and ESI-MS/MS. Each sample (5 µl) was loaded on a conductive pipette tip and directly infused using a chip-based Triversa Nanomate ESI system (Advion, Ithaca, NY) that was connected to the linear ion trap LTQ-XL (ThermoFinnigan, Somerset, NJ) mass spectrometer. The ESI source was operated at 1.5 kV electrospray voltage in the positive mode. Full scan mass spectra were acquired in the mass range of *m/z* 0-500. ESI-MS/MS analysis was performed by collision induced dissociation of the precursor ion of allicin m/z 163. The fragmentation pattern of the product ions from ESI-MS/MS of allicin from GE and standard were compared for further confirmation of identification of allicin in the GE.

Dynamic light scattering and zeta potential measurements:

Particle size distribution of GE-np-control and GE-np was determined by dynamic light scattering (DLS) method. Briefly, 1 mg/ml particles were suspended in PBS and measurements made on a DLS instrument, Dynapro Nanostar from Wyatt Technology (Santa Barbara, CA). The zeta potential was measured using a Malvern Zetasizer, Nano-ZS system (Malvern, MA)

Results and Discussion:

Size distribution and zeta potential of GE-np:

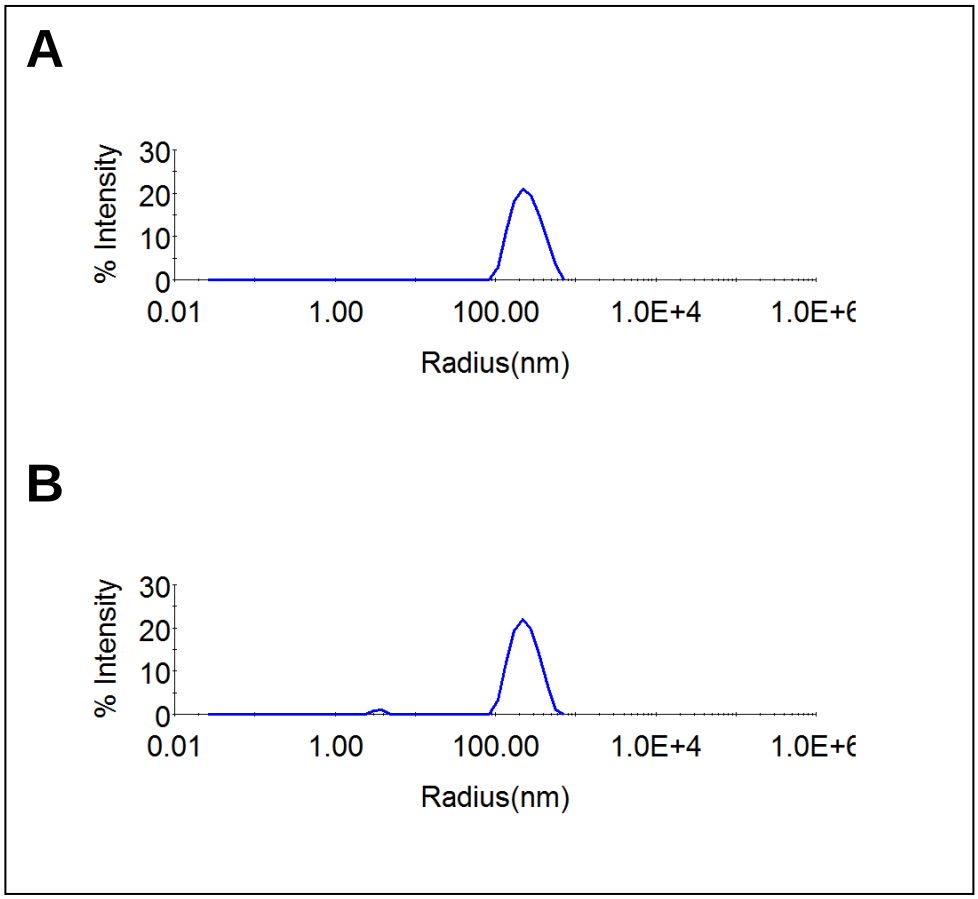

Figure S1. Particle size distribution of A) GE-np-control and B) GE-np. Particles were suspended at 1 mg/ml in PBS and the size distribution was measured using a dynamic light scattering instrument

Owing to their significance in governing the applicability of nanoparticles in drug delivery, we determined the size distribution, polydispersity index (PDI) and zeta potential of GE-np-control and GE-np. [7] The hydrodynamic radius of both the particles, ranged between 200 to 500 nm with the average size determined as 262 nm for GE-np-control (PDI - 0.017) and 245 nm (PDI - 0.015) for GE-np, respectively, indicating that the samples are not too heterogeneous. Such kind of heterogeneity in size is common for silica-based nanoparticles. [8, 9] Further, we determined the zeta potential of GE-np-control and GE-np as -21 and -22 mV, respectively. As described in the methods, the sol-gels were made from hydrolysis of TMOS and subsequent polymerization that generates free silanols. When the sol-gel is dried and ground into fine particles, the surface is left with some free silanols which may have contributed to the negative zeta potential of GE-np-control. [10] The GE-np preparation used a trace amount of APTS in addition to TMOS (20 ul of APTS for 3 ml of TMOS). APTS carries an amino group, which is expected to carry a positive charge under the conditions of zeta potential determination (PBS, pH 7.4). However, the zeta potential of GE-np is comparable to that of GE-np-control. Since only trace amount of APTS is used, not enough amino groups seem to be on the surface of the particles to make significant contribution towards zeta potential.

Supplementary references:

1. Friedman AJ, Blecher K, Schairer D, Tuckman-Vernon C, Nacharaju P, Sanchez D, et al. Improved antimicrobial efficacy with nitric oxide releasing nanoparticle generated S-nitrosoglutathione. *Nitric Oxide* 2011;**25**:381-86.

2. Schairer D, Martinez LR, Blecher K, Chouake J, Nacharaju P, Gialanella P, et al. Nitric oxide nanoparticles: pre-clinical utility as a therapeutic for intramuscular abscesses. *Virulence* 2012;**3**:62-7.

3. Santhosha SG, Jamuna P, Prabhavathi SN. Bioactive components of garlic and their physiological role in health maintenance: A review. *Food Bioscience* 2013;**3**:59-74.

4. Iciek M, Kwiecien I, Wlodek L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen* 2009;**50**:247-65.

5. Maldonado PD, Chanez-Cardenas ME, Pedraza-Chaverri J. Aged garlic extract, garlic powder extract, S-allylcysteine, diallyl sulfide and diallyl disulfide do not interfere with the antibiotic activity of gentamicin. *Phytother Res* 2005;**19**:252-4.

6. Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. *Phytother Res* 2003;**17**:97-106.

7. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lepidic nanocarrier systems. *Pharmaceutics* 2018;**10**.

8. Kaasalainen M, Aseyev V, von Haartman E, Karaman DŞ, Mäkilä E, Tenhu H, et al.. Size, Stability, and Porosity of Mesoporous Nanoparticles Characterized with Light Scattering. *Nanoscale Res Lett* 2017;**12**:74.

9. Lu Z, Owens H. A method to improve the quality of silica nanoparticles (SNPs) over increasing storage durations. *J Nanopart Res* 2018;**20**:213.

10. Vallet-Regi M, Tamanoi F. Overview of Studies Regarding Mesoporous Silica Nanomaterials and Their Biomedical Application. *Enzymes* 2018;**43**:1-10.